Reference: *Biol. Bull.* **187:** 208–214. (October, 1994)

# Homeoviscous Properties Implicated by the Interactive Effects of Pressure and Temperature on the Hydrothermal Vent Crab *Bythograea thermydron*

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**Abstract.** Specimens of the hydrothermal vent crab *Bythograea thermydron*, collected from 13° N on the East Pacific Rise, were exposed to pressures greater than those in their natural habitat over a range of temperatures to assess how increased hydrostatic pressure affects a species that requires high pressure to survive. We measured heart beat frequency and contraction waveform at pressures ranging from 28 MPa (normal environmental pressure for this species) to 62 MPa at 5°, 10°, and 20°C. At 5°C, increased hydrostatic pressure induced bradycardia or acardia in conjunction with marked disruption of the ventricular contraction waveform. The animals did not survive following decompression. The effects of increased pressure were less pronounced at 10°C and almost negligible at 20°C. Our results support previous findings at subambient pressures which suggest that the lipid bilayers of cell and organelle membranes are the primary sites affected by short-term pressure variation in deep-sea organisms. We also found evidence of an adaptive mechanism of pressure-temperature interaction in these animals from the eurythermic habitat of the hydrothermal vents.

## Introduction

Hydrostatic pressure increases by one atmosphere (101.3 kPa) for each 10 m of depth in the ocean; thus, organisms living at greater depths experience greater pressures (Saunders and Fofonoff, 1976). Elevated pressures have been shown to have a variety of effects on biological systems, with the primary loci for effects being enzyme and membrane systems (Macdonald and Miller, 1976; Wann and Macdonald, 1980; Siebenaller and Somero, 1989; Somero, 1992). At the enzymatic level, pressure exerts an inhibitory effect when there is a volume increase associated with substrate binding or catalytic activity (Siebenaller and Somero, 1989; Somero, 1992). In membranes, the structure of the membrane is altered by the greater compressibility of lipid as compared to water and other membrane components (Macdonald and Miller, 1976).

Both enzyme and membrane adaptations have been found in deep-sea organisms. The enzymatic adaptations involve a reduction in the volume change that occurs in the enzymes of deep-sea organisms during catalysis. As a result, enzyme-substrate affinity and other catalytic properties of the enzymes of deeper-living species are more independent of pressure (Siebenaller and Somero, 1989; Somero, 1992). At the membrane level, the adaptations involve a shift in membrane lipid composition with increasing depth. This shift maintains optimal fluidity (homeoviscous adaptation) in the face of increasing pressure, which would tend to order the membrane lipids (Cossins and Macdonald, 1984, 1986, 1989; Cossins and Sinensky, 1986). The effects of homeoviscous adaptation are expected to be especially prominent in excitable tissues and, unlike those of enzymatic adaptation, to show profound interaction with temperature: lower temperatures ameliorate the effects of lower pressures and higher temperatures ameliorate the effects of higher pressures through their opposite effects on the fluidity of membrane lipids (Cossins and Macdonald, 1989). In addi-

Received 7 December 1993; accepted 18 July 1994.

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tion, one would not expect homeoviscous adaptation to reduce the sensitivity to pressure, as is the case for enzymatic adaptations, but rather to shift the tolerated range of pressure in the same way that it does in temperature adaptation (Sinensky, 1974). There is some indication that the membranes of fishes living at depths greater than 2500 m have reduced compressibility (Behan *et al.*, 1992). Along with reducing the effects of pressure variation on membrane function, this adaptation would preserve the activity of membrane-associated enzymes which are critically dependent on the physical state of the membrane (Gibbs and Somero, 1989).

Although it is clear from the work cited above that both enzyme and membrane adaptations are found in deep-sea organisms, the evaluation of their relative importance must come from studies of the functioning of deep-sea animals. Much work on hydrostatic pressure effects has been concerned with the effects of elevated pressures on organisms that normally live at 1 atm. Other pressure studies have been concerned with species of fishes and crustaceans that live at depths of about 1000 m and have typically demonstrated mild responses to reduced pressure in conjunction with somewhat greater tolerance of elevated pressures (Belman and Gordon, 1979; Quetin and Childress, 1980). Recent studies that used pressure traps to recover specimens demonstrated that species living below 2000 m are dependent on elevated pressures (Macdonald and Gilchrist, 1980, 1982; Yayanos, 1981). Animals living at depths of about 2500 m around hydrothermal vents on the Galapagos Rift also require high pressure for long-term survival, although many can survive decompression to 1 atm if this is soon followed by recompression (Arp and Childress, 1981; Mickel and Childress, 1982a,b; Arp *et al.*, 1984). These animals are also uniquely suited to studies of pressure-temperature interactions because, unlike other deep-sea animals, they experience wide natural temperature ranges. Mickel and Childress (1982a) have demonstrated that when the vent crab *Bythograea thermydron* is exposed to pressures substantially lower than those of its natural habitat, heart rate is reduced and the EKG wave form becomes irregular. Furthermore, the onset of these effects was at higher pressures at higher temperatures and at lower pressures at lower temperatures, a result which the authors interpreted as evidence that membrane lipid properties are important in determining the pressure sensitivity of this species. This study did not, however, test for the effects of superambient pressures on this species, and no other such studies have been conducted on any deep-sea animal. The purpose of the present study was twofold: first, to determine at what pressures an elevation in pressure affects the functioning of a deep-sea animal that requires high pressure for survival; second, to examine the interactive effects of these elevated pressures with temperature.

## Materials and Methods

### *Collection and maintenance*

Baited traps deployed and retrieved by the Deep Submergence Vehicle *Alvin* were used to collect specimens of *B. thermydron*, of either sex, from the hydrothermal vent field at 13° N on the East Pacific Rise. Crabs were brought to the surface in insulated containers that kept temperature, but not pressure, relatively constant. Once at the surface, crabs were examined to obtain demographic data, then placed in steel vessels that were maintained at a pressure of 21 MPa (204 atm) and supplied with flowing seawater at approximately 8°C. Under such conditions, survival for more than 18 months has been reported (Mickel and Childress, 1982a).

### *Animal preparation*

In preparation for experimental use, crabs were slowly depressurized, removed from the maintenance vessel, and transferred to a shallow glass container filled with ice-cold seawater. Instrumentation of the animals for impedance pneumography was accomplished as quickly as possible to minimize disturbance and hypobaric trauma.

To measure heart beat frequency, holes were drilled in the dorsal carapace on either side of the heart and two fine silver wires were inserted so that their ends just penetrated the pericardial sinus. To prevent bleeding and hold the wires in position, dental dam was placed over the wires and holes and fixed to the carapace with cyanoacrylate cement. Changes in impedance associated with ventricular contraction were detected by an impedance converter (UFI, model 2991) and recorded on a Gould two-channel pen recorder.

Although not reported as a part of this study, the frequency of scaphognathite beating was recorded as an indicator of the physiological state of the animals. Two impedance pneumography electrodes were affixed inside the exhalant channel of the right branchial chamber and impedance changes caused by movement of the scaphognathite were recorded as above.

Following preparation, crabs were placed without additional restraint in a 6-l stainless steel vessel (Autoclave Engineers) filled with aerated, filtered seawater. Experimental temperatures were maintained inside the pressure vessel by a water-jacket surrounding the chamber. A hand pump (Enerpac) connected *via* Hastelloy C tubing and Autoclave Engineers fittings was used to pressurize the vessel to 28 MPa (272 atm); pressure was monitored using a gauge on the pump. The entire system was rated to 69 MPa (680 atm) working pressure with a 4:1 safety factor.

Animals were allowed to recover at normal environmental pressure until the heart beat frequency stabilized and the scaphognathite began to alternate between periods of continuous beating and inactivity in the fashion typical of nonstressed decapod crustaceans (McMahon and Wilkens, 1972). Animals that did not regain typical, stable readings of heart and scaphognathite activity within 2 h of instrumentation were not used in subsequent experiments.

*Experimental protocol*

Once the crabs appeared to have recovered, the chart speed was increased to allow resolution of individual contractions of the ventricle. Heart beat frequency was obtained by counting the waveform peaks over a 30-s period. Ventricular contraction pattern was quantified by calculating the standard deviation of 10 successive interbeat intervals. Immediately after this control recording, the pressure inside the experimental chamber was increased from 28 to 31 MPa. The animals were allowed 10 min to adjust, then the chart speed was increased for determination of ventricular contraction frequency and pattern before the pressure was raised to 34 MPa. This protocol continued, with pressure increasing in 3-MPa steps, until the maximum pressure tested (62 MPa) was reached. After 30 min, the vessel pressure was gradually lowered to 28 MPa. Data were obtained 30 min after return to the original pressure, and the chamber was subsequently depressurized for removal of the crabs.

The effects of pressure and temperature on heart beat frequency and contraction pattern were estimated using analysis of variance with repeated measures (ANOVAR). ANOVARs significant at the 0.05 level were further analyzed using Tukey's HSD multiple-comparison test. In addition, Spearman rank correlation was used to determine the relationship between pressure and heart beat frequency at each temperature. All values are reported as the mean ± 1 SEM.

## Results

*Contraction frequency*

The control heart beat frequency of crabs allowed to recover from the instrumentation procedure was positively correlated with experimental temperature ($r_s = 0.937$, $n = 15$, $P < 0.01$). The relationship between temperature and contraction frequency did not, however, remain constant as pressure within the experimental chamber increased (Fig. 1).

At 5°C, heart rate dropped 92% from its control value at 28 MPa to 3.3 ± 1.3 $min^{-1}$ after 10 min at 62 MPa (Fig. 1). The negative correlation between contraction frequency and pressure was highly significant ($r_s = -0.940$, $n = 60$, $P < 0.01$). Heart beat frequency was significantly different from the control value at all pressures greater than 41 MPa at this temperature ($F = 72.05$, $P < 0.01$). Following 30 min of recovery at 28 MPa, heart rate was still markedly different from the pre-exposure value ($P < 0.01$; Table 1).

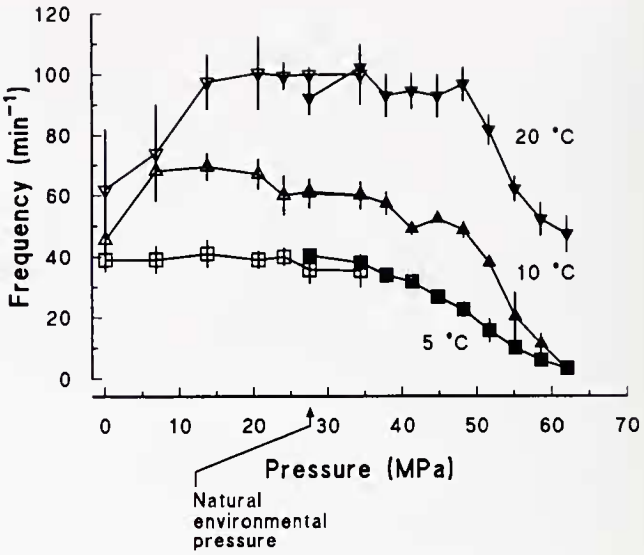


**Figure 1.** The effect of altered pressure on heart beat frequency in *Bythograea thermydron* at three temperatures. For comparison, the data of Mickel and Childress (1982a; open symbols) are presented along with those from the present study (solid symbols). The middle curve from the former study was obtained from experiments conducted at 12° rather than 10°C. At 5°C (■) the rate fell almost linearly to its minimum value as pressure was increased; however, contraction frequency was maintained until the pressure reached about 52 MPa in crabs tested at 10°C (▲). The heart rate of crabs tested at 20°C (▼) also remained constant at pressures below 52 MPa, but then declined less severely than in 10°C crabs. Generally, the decline in heart rate brought about by hypobaric exposure was ameliorated by lower temperatures, whereas high temperatures buffered the effects of hyperbaric exposure. Although the crabs used in these two studies were obtained from sites thousands of miles apart and the experimental techniques varied somewhat in detail, excellent agreement of the normobaric data supports the validity of combining the data sets. Spearman rank correlation coefficients ($r_s$) for heart rate and pressure (data from the present study) were calculated as: $r_s = -0.940$, $n = 60$, $P < 0.01$ at 5°C; $r_s = -0.923$, $n = 30$, $P < 0.01$ at 10°C; $r_s = -0.706$, $n = 60$, $P < 0.01$ at 20°C. Normal environmental pressure is about 28 MPa for this species. Data shown as mean ± 1 SEM.

The heart beat frequency of crabs tested at 10°C fell from 60.7 ± 4.8 to 3.0 ± 1.5 $min^{-1}$ over the same pressure range as above (Fig. 1). The correlation between pressure and heart rate ($r_s = -0.923$, $n = 30$, $P < 0.01$) was also significant, but the difference between control and experimental contraction frequencies was significant only at pressures greater than 52 MPa ($F = 32.76$, $P < 0.05$). The bradycardia continued immediately after the return to normal environmental pressure ($P < 0.05$), but after 30 min of recovery the heart rate had rebounded and

**Table I**

*Heart rate of* Bythograea thermydron *before and after hyperbaric exposure (62 MPa) at three temperatures*

| | $f_H$(min$^{-1}$) | |
|---|---|---|
| Temperature (°C) | Control | Recovery |
| 5 (6) | 40.5 ± 0.8 | 15.2 ± 4.0* |
| 10 (3) | 60.7 ± 4.8 | 43.3 ± 13.0 |
| 20 (6) | 92.2 ± 5.5 | 104.2 ± 9.6 |

Crabs were allowed 30 min after return to normal pressure before the recovery values were obtained. Values are shown as the mean ± 1 SEM; sample size in parentheses.

* Value significantly different from control ($P < 0.01$, ANOVAR).

was not significantly different from the control value (Table I).

At 20°C, the change in heart rate with increasing pressure was much less dramatic than at the lower temperatures. The rate decreased only 49%, reaching a minimum of 47.3 ± 5.9 min$^{-1}$ at 62 MPa (Fig. 1). There was a significant correlation between heart beat frequency and pressure ($r_s = -0.706$, $n = 60$, $P < 0.01$), but significant changes from the control rate occurred only above 55 MPa ($F = 20.38$, $P < 0.01$). As soon as the pressure was lowered, heart beat frequency rebounded above pre-exposure values and remained elevated for at least 30 min (Table I).

*Contraction pattern*

The relationship between ventricular contraction pattern and experimental pressure was also dependent on temperature. Generally, the systolic spikes became less defined and the interbeat interval tended to vary more as pressure was increased. These effects were more pronounced at lower temperatures (Figs. 2, 3). Crabs tested at 5°C showed increased variability in ventricular contraction waveform at pressures as low as 34 MPa; variability continued to increase until about 55 MPa, when ventricular contractions became extremely sporadic or ceased. At 62 MPa, the standard deviation of the interbeat intervals increased significantly compared to the control value ($F = 20.14$, $P < 0.01$; Fig. 3). Following return to normal environmental pressure there was little increase in the uniformity of ventricular contraction and all crabs died within 1 h of decompression, suggesting that the effects of hyperbaric exposure are irreversible at this temperature.

At 10°C, the ventricular contraction pattern began to degenerate at pressures above 41 MPa (Fig. 2). The higher chart speed used at this temperature allows resolution of biphasic contraction peaks and ventricular fibrillation at pressures of 55 and 62 MPa, respectively. Variability of the interbeat interval also increased significantly ($F = 17.54$, $P < 0.01$) compared to the 28 MPa control (Fig. 3). After 30 min of recovery at 28 MPa, the ventricular contraction pattern became much more regular, although a diastolic notch not present in the control recording became evident (Fig. 2), and the standard deviation of the interbeat intervals was markedly reduced (Fig. 3). Crabs in this group were returned to normobaric holding vessels, but all died within 24 h of experimental use.

Above 55 MPa the interbeat interval of crabs tested at 20°C became increasingly variable (Fig. 3) and there was evidence of a systolic plateau in some animals (Fig. 2). However, all animals examined at this temperature maintained organized cardiac activity, and they regained their initial ventricular contraction pattern as soon as they were returned to normal environmental pressure. All animals in this group survived, apparently healthy, for at least 48 h after experimental use.

## Discussion

During preparation at ambient sea-level pressure the crabs appeared healthy and responsive, although all showed the marked lack of coordination typical of this species when depressurized (Mickel and Childress, 1982a). Tolerance of repeated decompression for extended periods and survival at sea level at 5°C for up to 5 days have also been reported (Mickel and Childress, 1982a), suggesting that the short hypobaric exposure required for instrumentation of the crabs did not irreversibly affect their physiological state.

The relationship between temperature and heart beat frequency at the normal environmental pressure for *B. thermydron* is directly comparable to that observed in shallow-living invertebrates (deFur and Mangum, 1979). At higher pressures this relationship changed markedly, with heart rate first becoming more, then less dependent on temperature in the 5 to 10°C range. Between 10 and 20°C, the dependency of heart beat frequency on temperature remained more or less constant over the entire pressure range.

The effect of high hydrostatic pressure on membrane lipids is to decrease fluidity by increasing the order of the hydrophobic acyl chains within the membrane (Wann and Macdonald, 1980; Somero, 1992). This packing of the acyl chains may both change the ion permeability of the membrane and limit the diffusion of membrane proteins (Cossins and Macdonald, 1989). In addition, fusion of neurotransmitter vesicles with the postsynaptic terminal of nerve fibers may be disrupted (Wann and Macdonald, 1980), leading to failure of postsynaptic activation (see below). The combined tendencies of low temperature and high pressure to order membrane lipids may be countered

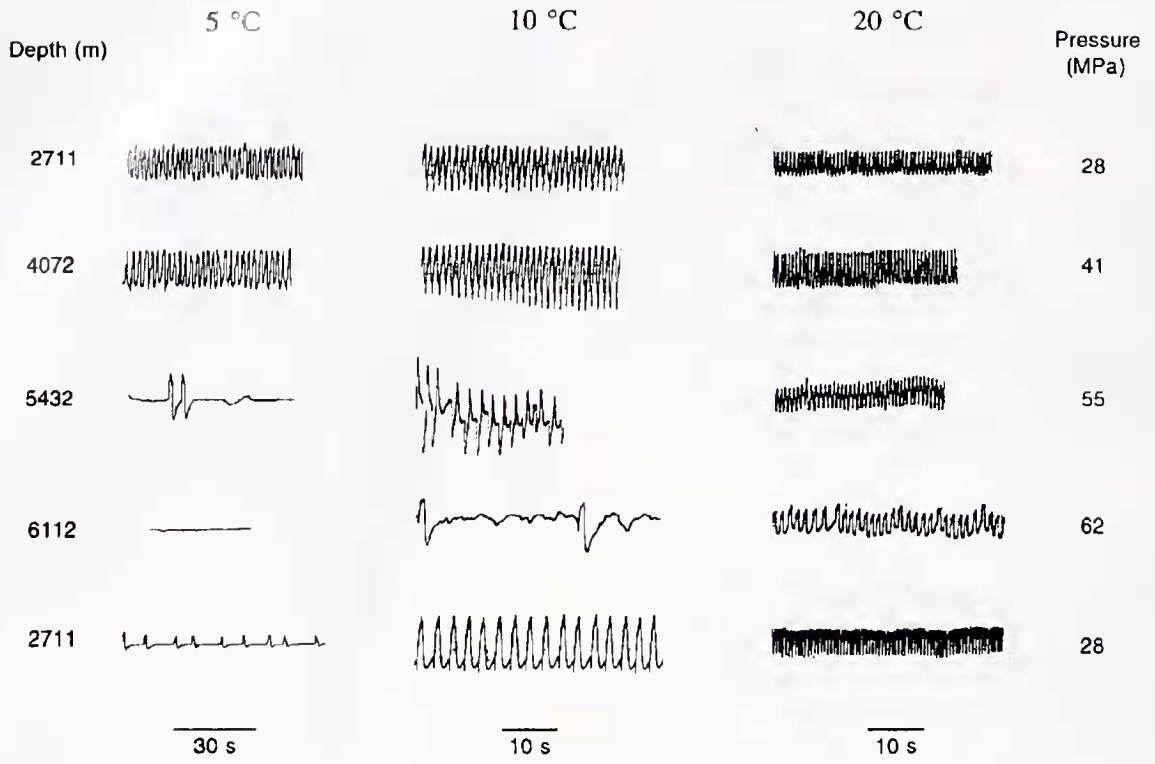


**Figure 2.** Chart records showing ventricular contraction in three individual *Bythograea thermydron* during progressive hyperbaric exposure at 5°, 10°, and 20°C. The 28 MPa records at the bottom of the illustration were obtained following 30 min of recovery at normal environmental pressure for this species. At 5°C, the contraction waveform became less distinct and interbeat variability increased with higher pressure. Total acardia occurred at pressures higher than 55 MPa, and contractions remained disorganized following return to normal pressure. Hyperbaric exposure was better tolerated in crabs tested at 10°C, but extra systolic spikes and ventricular fibrillation were evident at higher pressures. Recovery was more complete at this temperature than at 5°C, although the contraction frequency remained depressed. Compared to the other two experimental temperatures, the effects of increased pressure on contraction frequency and waveform at 20°C were minimal. After 30 min of recovery, the contraction pattern had returned to normal and the heart rate had rebounded above control values.

within the lifetime of an organism by increasing the ratio of unsaturated to saturated fatty acids composing the membrane bilayer (Cossins and Macdonald, 1989). In organisms living in the high pressure–high temperature environment of the hydrothermal vents, the effects of high pressure on membrane fluidity may be partially compensated, requiring a lower proportion of unsaturated membrane constituents to achieve optimal membrane viscosity.

The beat of the crustacean heart is neurogenic (Alexandrowicz, 1932), generated by burst discharges from the cardiac ganglion (CG) on its inner dorsal wall. The striated muscle of the myocardium is polyneuronally and polysynaptically innervated by processes from the large motor neurones of the CG (Alexandrowicz, 1932). This innervation, along with tight electrotonic coupling between individual muscle fibers, ensures rapid spread of excitation throughout the myocardium, resulting in strong, coordinated contraction of the ventricle with each burst from the CG (Kuramoto and Kuwasawa, 1980). The amplitude and duration of spontaneous burst discharges from these motoneurons may be modulated by output from the small pacemaker cells at the posterior of the ganglion (Hartline, 1967), although it has been suggested that fine control of burst characteristics is achieved by interaction between the large and small CG cells (Sullivan and Miller, 1984). In the gastropod *Helix*, increased hydrostatic pressure reduces the firing rate of postsynaptic cells (Wann *et al.*, 1979), possibly due to a reduction in excitatory junctional potential (ejp) amplitude arising from decreased neurotransmitter release at higher pressures. Although pacemaker cell burst frequencies may increase in response to increased pressure (Wann and Macdonald, 1980), there are several potential sites for postsynaptic failure between the pacemaker cells of the crustacean CG and the myocardium.

Mickel and Childress (1982a) showed that both the reduction in heart beat frequency and the degeneration of EKG organization brought about by decreasing pressure

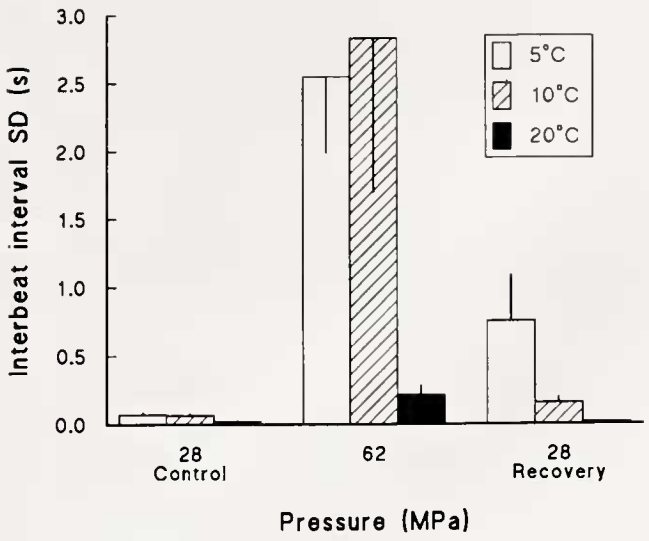


**Figure 3.** Average standard deviation of the intervals between 10 successive contractions of the ventricle of *Bythograea thermydron* before, during, and after hyperbaric exposure at 5°, 10°, and 20°C. Interbeat variability increased markedly in crabs exposed to 62 MPa pressure at both 5° and 10°C ($F = 20.14$, $P < 0.01$ and $F = 17.54$, $P < 0.01$, respectively); however, variability remained much higher after return to normal pressure in crabs tested at the lower temperature. The interbeat interval also increased significantly in response to hyperbaric exposure at 20°C ($F = 8.42$, $P < 0.01$), although the change was much less dramatic and recovery more complete than at 5° or 10°C. Error bars represent 1 SEM.

were less pronounced at lower temperatures. The change in heart rate accompanying decompression was, in fact, negligible at 5°C (Mickel and Childress, 1982a; Fig. 1). In contrast, the present study clearly demonstrates that higher temperatures reduce the severity of bradycardia resulting from hyperbaric exposure as well as limit the associated disorganization of the contraction waveform. If the primary effects of pressure variation on cardiac function were enzymatically mediated, then one would expect that temperature changes would have comparable effects on cardiac function under both hypo- and hyperbaric conditions. Conversely, if the system affected by pressure challenge is lipid-based, then a decrease in pressure (which decreases the viscosity of phospholipid membranes) would be compensated by a temperature reduction (causing an increase in membrane viscosity), whereas increased pressure would be counteracted by a temperature increase. The latter type of pressure-temperature interaction has now been shown to occur in response to both hypo- and hyperbaric exposure in *B. thermydron*, implicating a homeoviscous mechanism in which phospholipid membrane fluidity is affected by external pressure disturbance. Superfluous ventricular contraction spikes and prolonged plateaus suggest that disturbed membrane transport leads to the failure of neurotransmitter release and (or) myocardial excitation in the neuromuscular system of the heart.

The failure of crabs subjected to a pressure of 62 MPa at 5°C to recover fully from treatment suggests that disruption of the phospholipid membranes at this pressure-temperature combination is so complete as to be irreversible. The slower rate of recovery of crabs tested at 10°C and the complete recovery of animals in the 20°C group indicates that the higher temperatures precluded complete disruption of the membrane systems and further supports the involvement of membrane properties rather than enzyme kinetics in the observed cardiac responses to changes in pressure.

Shedding of proteins from cell membranes begins at pressures above 30 MPa (Deckmann *et al.*, 1985), and is another possible explanation for the irreversible effects of hyperbaric exposure observed in *B. thermydron*. Substantial release of integral membrane proteins occurs only at much higher pressures (about 100 MPa), however, and protein release is enhanced by higher temperatures (Deckmann *et al.*, 1985). These findings are in contrast to the present data, which show more deleterious effects of increased pressure at lower temperatures.

Despite the changes in heart beat frequency and ventricular contraction pattern associated with pressure variation in *B. thermydron*, this species shows remarkable tolerance toward pressure changes at all temperatures tested. At 5°C, the lowest temperature used in the present study, there were no significant differences in heart rate and no obvious changes in contraction pattern at pressures up to 41 MPa. Previous results from the same species at 5°C showed no significant pressure effect on heart rate between 0.1 and 34 MPa and disruption of the EKG waveform only at pressures less than 6.9 MPa (Mickel and Childress, 1982a), giving *B. thermydron* a tolerable pressure range of 6.9 to 41 MPa for apparently normal physiological function. At the higher temperatures tested, the pressure range for stable cardiac function is shifted toward higher pressures, but the magnitude of the tolerable range remains similar. The tolerance of *B. thermydron* for such broad ranges of pressure at any temperature implicates adaptations that decrease membrane compressibility and is consistent with both the prediction of Gibbs and Somero (1989) and the findings of Behan *et al.* (1992) that species found at greater depths show greater compensation for the effects of pressure. A reduction in membrane compressibility compared with shallower-living species would preserve the integrity and function of cell membranes and their associated proteins, making this deep-sea crab less susceptible to the deleterious effects of changes in environmental pressure.

**Acknowledgments**

The authors thank the captains and crews of R/V *John V. Vickers* and R/V *Atlantis II*, and the pilots of DSV

*Alvin;* without them these studies could not have been conducted. Special thanks are also due to Dr. G. Somero for his counsel on the effects of pressure on enzyme function, and L. Gorodezky for her assistance and support during the experimental portion of this work. Financial support was provided by NSF grants OCE-9012076 and OCE-9301374 as well as by NSERC and AHFMR fellowships to CNA.

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